TOOLS IN BRIEF

SENSORS AND PROBES

A red calcium indicator dye for dual-color imaging

Despite the advent of genetically encoded calcium indicators, calcium dyes remain indispensable for a variety of neuroscience imaging applications, especially in the red spectrum. Collot *et al*. developed a red calcium indicator called CaRuby-Nano, which is part of a larger family of dyes based on X-Rhodamine and the calcium-binding moiety BAPTA. CaRuby-Nano's sensitivity is in the nanomolar range and therefore similar to that of established green calcium indicators. This high affinity makes the dye most suitable for the detection of small calcium changes or single action potentials. CaRuby-Nano can be used for functional imaging *in vivo* or *in vitro*: for example, using two-photon imaging. Furthermore, the dye can be combined with genetically encoded reporters that emit in the green spectrum, which Collot *et al*. demonstrate in the mouse olfactory bulb.

Collot, M. *et al. eLife* **4**, e05808 (2015).

MOLECULAR BIOLOGY

Enter the diatom

Diatoms, the beautiful, kaleidoscopic algae that make up a significant fraction of the world's phytoplankton, are important primary producers with potential as biofuels. Yet the study of these unique creatures, which are mainly unicellular and encased in silica walls, is hindered by poor genetic tools. Karas *et al*. now describe the first stable plasmid vector for diatoms. The plasmid contains a centromeric fragment from yeast that allows it to segregate into daughter cells with near-perfect efficiency. The authors use it to express proteins from the diverse diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. To transfer the episomes, they recruit *Escherichia coli*, providing the first evidence that bacteria can carry out cross-kingdom conjugation with diatoms and showing that it can be an effective means to deliver foreign DNA to these organisms.

Karas, B.J. *et al. Nat. Commun.* **6**, 6925 (2015).

SENSORS AND PROBES

Bright cyan and orange probes for bioluminescence imaging

Bioluminescence is widely used in cell-based assays and for *in vivo* imaging. However, naturally bioluminescent proteins such as luciferases suffer from limitations including low brightness. To overcome this problem, researchers created a protein called Nano-lantern by fusing a mutant version of *Renilla* luciferase (Rluc) to the yellow fluorescent protein Venus. In the presence of luciferase substrate, the emitted light from Rluc is transferred by bioluminescence resonance energy transfer to Venus, yielding bright yellow emitted light. Takai *et al*. now extend this work to generate bright cyan and orange probes called cyan Nano-lantern and orange Nano-lantern. For cyan Nano-lantern, Venus from the original Nano-lantern was replaced with mTurquoise; for orange Nano-lantern, it was replaced with mKusabiraOrange2. These probes are ~20-fold brighter than wild-type Rluc and enable enhanced multicolor bioluminescence imaging.

Takai, A. *et al. Proc. Natl. Acad. Sci.* **112**, 4352–4356 (2015).

MODEL ORGANISMS

Pre- and postsynaptic activity reporters

Genetically encoded calcium indicators are popular tools for monitoring neural activity in *Drosophila*. However, these sensors are usually expressed throughout the neuron and do not report localized calcium signals. Pech *et al*. generated calcium sensors that are targeted to pre- or postsynaptic compartments in *Drosophila*. The researchers fused the calcium sensor GCaMP3 either to the postsynaptically localized protein *d*Homer or to synaptophysin, which resides in vertebrate presynapses and localizes correctly when expressed in *Drosophila*. Furthermore, fusing synaptophysin to the pH sensor pHTomato yielded a reporter for synaptic vesicle fusion. The researchers applied these tools to analyze synaptic plasticity in the *Drosophila* olfactory system using two-photon imaging.

Pech, U. *et al. Cell Rep.* **10**, 2083–2095 (2015).